

WHAT IS CLAIMED:

1. A method of performing an expression microarray to determine the presence of a target, comprising:

5 (a) attaching a probe which will recognize a target to a polymer-coated support by a [2 + 2] photocycloaddition to form a microarray;

(b) contacting an aqueous target solution with the microarray, for a time sufficient to form a complex between complementing targets and probes, wherein the target solution comprises an aqueous buffer solution and the target; and

(c) scanning the microarray to determine the presence of the target.

2. The method of claim 1, further comprising application of a probe standard to the polymer-coated support.

15 3. The method of claim 2, wherein the probe and probe standard are applied to the polymer-coated support in about equal amounts, on a weight basis.

4. The method of claim 2, wherein said aqueous target solution further comprises a target standard.

20 5. The method of claim 1, wherein the concentration of the target is determined through comparison of the fluorescence intensities of the target and target standard.

25 6. The method of claim 4, wherein the target standard is selected from the group consisting of yeast mRNA and bacterial mRNA, or combinations thereof.

0950250 05282560

7. The method of claim 1, wherein scanning occurs in a spectrometer capable of measuring and recording fluorescence intensity and position.

8. The method of claim 1, wherein the aqueous target solution comprises a buffer capable of maintaining pH from about 6 to 9.

9. The method of claim 1, wherein the target is a labeled nucleic acid.

10. The method of claim 9, wherein the label is selected from the group consisting of Cy-3, Cy-5, Cy-5.5, and ALEXA FLUOR.

11. The method of claim 9, wherein the label is Cy-3.

12. The method of claim 9, wherein the labeled nucleic acid is mRNA, RNA, DNA, amplified RNA, amplified DNA, or modifications thereof.

13. The method of claim 9, wherein the labeled nucleic acid is mRNA, RNA, or DNA.

14. The method of claim 1, further comprising developing of the microarray after application of the target solution.

15. The method of claim 14, wherein developing lasts from 1 minute to 42 hours.

16. The method of claim 14, wherein developing lasts about 16 hours.

17. The method of claim 14, wherein developing occurs between 30 and 45° C.

18. The method of claim 14, wherein developing occurs at about 37°
C.

19. The method of claim 14, further comprising washing with an
aqueous wash after developing.

20. The method of claim 19, wherein the aqueous wash contains a
buffer capable of maintaining pH from about 6 to 9.

21. The method of claim 20, wherein the buffer comprises
phosphate and sodium chloride.

22. The method of claim 1, wherein the solid support is a material
selected from the group consisting of nylon, polystyrene, glass, latex,
polypropylene, and activated cellulose, or combinations thereof.

23. The method of claim 1, wherein the solid support is glass.

24. The method of claim 1, wherein the polymer is a polymer,
reactive prepolymer, or copolymer made of at least two co-monomers
wherein at least one of said co-monomers can undergo [2 + 2]
photocycloaddition.

25. The method of claim 24, wherein the polymer or reactive
prepolymer contains polyacrylamide.

26. The method of claim 1, wherein the polymer is a polymer,
reactive prepolymer, or copolymer chemically modified to contain a
reactive group that undergoes [2 + 2] photocycloaddition.

27. The method of claim 26, wherein the polymer or reactive
prepolymer contains polyacrylamide.

28. The method of claim 1, wherein said probe comprises a nucleic acid fragment containing less than about 1000 nucleotides, and further optionally comprises a linker.

29. The method of claim 28, wherein said linker is an organic chain of about 6 to 100 atoms in length.

30. The method of claim 28, wherein said nucleic acid fragment is selected from the group consisting of synthetic nucleotides and modified nucleotides, or combinations thereof.

31. The method of claim 1, wherein said probe is cDNA.

32. The method of claim 1, wherein said probe is chemically modified to contain a reactive group that undergoes [2 + 2] photocycloaddition.

33. The method of claim 32, wherein said probe is chemically modified with a phosphoramidite.

34. The method of claim 33, wherein said phosphoramidite is chemically functionalized with a reactive site capable of undergoing [2 + 2] photocycloaddition.

35. The method of claim 33, wherein said phosphoramidite is functionalized with a cinnamide.

36. The method of claim 1, wherein said probe inherently contains a reactive site that undergoes [2 + 2] photocycloaddition.

37. The method of claim 1, wherein the reactive site present on the polymer and/or the reactive site present on the probe(s) contains an alkene group.

38. The method of claim 1, wherein the reactive site present on the polymer and/or the reactive site present on the probe is selected from the group consisting of dimethyl maleimide, maleimide, thymine, polythymine, acrylate, cinnamate, and citraconimide, or combinations thereof.

5 39. The method of claim 1, wherein the polymer coated support is a hydrogel microarray.

40. The method of claim 39, wherein the microarray is formed by crosslinking a hydrogel simultaneous with step (a).

41. The method of claim 39, wherein prior to step (a) the hydrogel microarray is prepared by first crosslinking a hydrogel.

42. The method of claim 1, wherein a photosensitiser is added during step (a).

43. The method of claim 42 wherein, the photosensitiser is Anthroquinone-2-sulfonic acid.

15 44. A method of performing a single nucleotide polymorphism microarray to determine the presence of a target, comprising:

(a) attaching a probe which will recognize a target to a polymer-coated support by a [2 + 2] photocycloaddition to form a microarray;

20 (b) contacting an aqueous target solution with the microarray, for a time sufficient to form a complex between complementing targets and probes, wherein the target solution comprises an aqueous buffer solution, the target, an active enzyme, and a labeled carrier; and

(c) scanning the microarray to determine the presence of the target.

45. The method of claim 44, further comprising application of a probe standard to the polymer-coated support.

46. The method of claim 45, wherein the probe and probe standard are applied to the polymer-coated support in about equal amounts, on a weight basis.

47. The method of claim 45, wherein said aqueous target solution further comprises a target standard.

48. The method of claim 44, wherein the concentration of the target is determined through comparison of the fluorescence intensities of the target and target standard.

49. The method of claim 47, wherein the target standard is selected from the group consisting of yeast mRNA and bacterial mRNA, or combinations thereof.

50. The method of claim 44, wherein scanning occurs in a spectrometer capable of measuring and recording fluorescence intensity and position.

51. The method of claim 44, wherein the aqueous target solution comprises a buffer capable of maintaining pH from about 6 to 9.

52. The method of claim 44, wherein the aqueous target solution comprises an active enzyme.

53. The method of claim 52, wherein the active enzyme is capable of transferring a label to a probe/target complex by single base extension.

54. The method of claim 52, wherein the active enzyme is thermosequanase.

55. The method of claim 44, wherein the aqueous target solution comprises a fluorescently labeled carrier.

56. The method of claim 55, wherein the fluorescently labeled carrier provides a transferable label to an active enzyme for transfer to a probe/target complex by single base extension.

57. The method of claim 55, wherein the fluorescently labeled carrier is di-deoxynucleotide triphosphate.

58. The method of claim 55, wherein the label is selected from the group consisting of Cy-3, Cy-5, Cy-5.5, and ALEXA FLUOR.

59. The method of claim 55, wherein the label is Cy-3.

60. The method of claim 44, wherein the target is a nucleic acid.

61. The method of claim 60, wherein the nucleic acid is mRNA, RNA, DNA, amplified RNA, amplified DNA, or modifications thereof.

62. The method of claim 60, wherein the nucleic acid is mRNA, RNA, or DNA.

63. The method of claim 44, further comprising developing of the microarray after application of the target solution.

64. The method of claim 63, wherein developing lasts for 30 to 60 heating/cooling cycles.

65. The method of claim 63, wherein developing lasts for 40 to 50 heating/cooling cycles.

66. The method of claim 63, wherein developing occurs between 30 and 70° C.

67. The method of claim 63, wherein developing occurs between 40 and 60° C.

68. The method of claim 63, further comprising washing with an aqueous wash after developing.

5 69. The method of claim 68, wherein the aqueous wash is performed between 40 and 70° C.

70. The method of claim 68, wherein the aqueous wash is performed between 50 and 60° C.

71. The method of claim 68, wherein the aqueous wash contains a buffer capable of maintaining pH from about 6 to 9.

72. The method of claim 71, wherein the buffer comprises phosphate and sodium chloride.

73. The method of claim 44, wherein the solid support is a material selected from the group consisting of nylon, polystyrene, glass, latex, polypropylene, and activated cellulose, or combinations thereof.

74. The method of claim 44, wherein the solid support is glass.

75. The method of claim 44, wherein the polymer is a polymer, reactive prepolymer, or copolymer made of at least two co-monomers wherein at least one of said co-monomers can undergo [2 + 2] photocycloaddition.

76. The method of claim 75, wherein the polymer or reactive prepolymer contains polyacrylamide.

77. The method of claim 44, wherein the polymer is a polymer, reactive prepolymer, or copolymer chemically modified to contain a reactive group that undergoes [2 + 2] photocycloaddition.

78. The method of claim 77, wherein the polymer or reactive prepolymer contains polyacrylamide.

79. The method of claim 44, wherein said probe comprises a nucleic acid fragment containing less than about 1000 nucleotides, and further optionally comprises a linker.

80. The method of claim 79, wherein said linker is an organic chain of about 6 to 100 atoms in length.

81. The method of claim 79, wherein said nucleic acid fragment is selected from the group consisting of synthetic nucleotides and modified nucleotides, or combinations thereof.

82. The method of claim 44, wherein said probe is cDNA.

83. The method of claim 44, wherein said probe is chemically modified to contain a reactive group that undergoes [2 + 2] photocycloaddition.

84. The method of claim 83, wherein said probe is chemically modified with a phosphoramidite.

85. The method of claim 84, wherein said phosphoramidite is chemically functionalized with a reactive site capable of undergoing [2 + 2] photocycloaddition.

86. The method of claim 84, wherein said phosphoramidite is functionalized with a cinnamide.

87. The method of claim 44, wherein said probe inherently contains a reactive site that undergoes [2 + 2] photocycloaddition.

88. The method of claim 44, wherein the reactive site present on the polymer and/or the reactive site present on the probe(s) contains an electron deficient alkene group.

89. The method of claim 44, wherein the reactive site present on the polymer and/or the reactive site present on the probe is selected from the group consisting of dimethyl maleimide, maleimide, thymine, polythymine, acrylate, cinnamate, and citraconimide, or combinations thereof.

90. The method of claim 44, wherein the polymer coated support is a hydrogel microarray.

91. The method of claim 90, wherein the microarray is formed by crosslinking a hydrogel simultaneous with step (a).

92. The method of claim 90, wherein prior to step (a) the hydrogel microarray is prepared by first crosslinking a hydrogel.

93. The method of claim 44, wherein a photosensitiser is added during step (a).

94. The method of claim 93 wherein, the photosensitiser is Anthroquinone-2-sulfonic acid.

092350 0801
104030 052210